Unexpected spin-offs

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In 1935 we of the recently established Subdivision of Biophysics at the Rockefeller Institute for Medical Research had decided that the time had come to move from the X-ray analysis of simple organic compounds towards an attempt to get data from substances of more immediate biological interest. We had chosen for diverse reasons collagen, hemoglobin and globulins in general. Alexis Carrel, with whom I was closely associated, remembered that some years earlier Nageotte and Guyon [1] had shown that immature rat-tail tendons, in contrast to mature connective tissues, could be solubilized by very dilute acetic acid and then re-constituted. We began studying the X-ray patterns of such collagens [2], of hemoglobin [3] and of other crystalline proteins (such as pepsin, insulin and the tryptic enzymes) which were just becoming available. Our interests centered around especially large globulins present in the antipneumococcal sera then in clinical use.

The recently perfected oil turbine ultracentrifuge of Svedberg had already proved invaluable for the characterization of a wide range of macromolecular substances (see this issue p. 117). It clearly could provide the information we needed but was quite beyond our financial reach. Beams [4] in Virginia had, however, been experimenting for several years with the air-driven spinning tops of the Belgian engineers Henriot and Huguenard [5] and had shown they could successfully rotate large objects suspended by a thin shaft and enclosed in a vacuum; he had constructed various types of ultracentrifuge.

Believing that ultracentrifugal control of the proteins we were investigating was essential, I decided to build a simplified analytical centrifuge based on this work of Beams. I had come to the Rockefeller Institute from the Geophysical Laboratory where a first class instrument shop was at the core of all research. The Rockefeller Institute had no shop but I had been able to include facilities in my department to make the various pieces of apparatus needed for our crystal structure investigations. This enabled us to undertake the design and development of an air-driven analytical ultracentrifuge able to operate up to the maximum speeds permitted by the strength of its rotors. Pickels, who had worked with Beams, had come to the laboratories of the Rockefeller Foundation with the object of constructing a centrifuge for work with yellow fever. We collaborated in the building of our first ultracentrifuge and in the investigation of light metals to replace the steel in its parts [6]. This substitution was desirable because much less energy would be required to spin the lighter metal and the instrument would thus be far safer. We obtained specially treated aluminum-magnesium billets from the Dow Chemical Company and developed rotors which could operate at nearly as high a speed as the strongest steel.

The first ultracentrifuge built in my shop had a steel rotor and was employed routinely for the measurement of sedimentation rates until it was wrecked one day when either a vacuum leak developed or the rotor failed. The vacuum chamber, of two-inch armor plate, was knocked into an egg shape, the turbine was driven through the ceiling of the room and the rotor was shattered. Fragments bounced several times around the room and one piece flew through a thick, wire-reinforced glass window without shattering it. The damage it caused left no doubt of the desirability of light metal rotors.

With our analytical ultracentrifuges we measured the sedimentation constants of a number of proteins including the pneumococcal antibody globulins [7]. I noticed during one of our runs that excellent crystals of hemoglobin formed in the bottom of the analytical cell. This immediately suggested that ultracentrifugation might not only concentrate our globulins but give an especially desirable way to obtain the protein crystals we were seeking for X-ray study. Quantity heads holding at least 100 ml were constructed of different alloys and used to purify, by differential centrifugation, the proteins with which we were then working. When the heads began to show evidence of corrosion or metal fatigue they were accelerated to bursting in an outdoor pit, providing in this way firm evidence of the relative merits of the alloys and the safe speeds at which they could be run.

While this was going on, Stanley came to New York to give a colloquium describing his chemical isolation of the tobacco mosaic virus. There was evidence that the virus was somewhat altered by the salting-out he used and it seemed to me that quantity ultracentrifugation offered a far less damaging way to concentrate viruses. Stanley gave me infectious plant juice which we then differentially sedimented to yield an especially pure product [8]. The result was so good that several other plant viruses, latent mosaic, tobacco ring spot, cucumber viruses, aucuba mosaic and to-

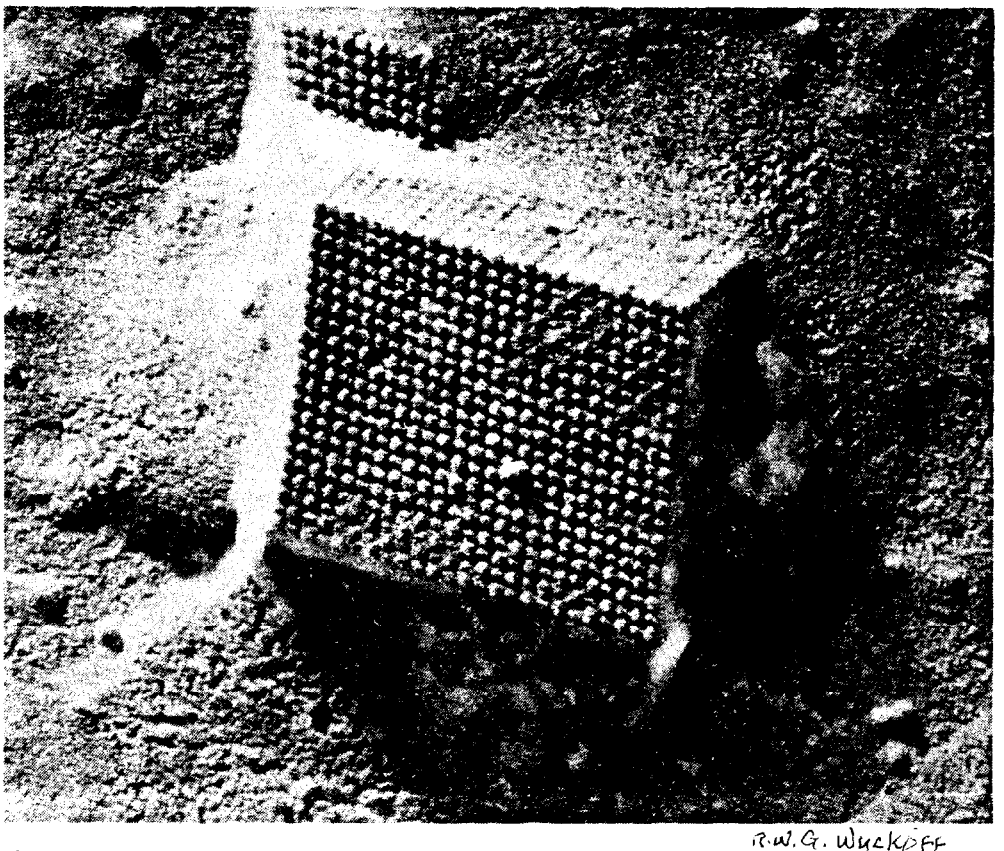

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Fig. 1. One of the first electron micrographs of tobacco necrosis virus crystals taken by ~~K.M. Smith~~ (×40000).

bacco necrosis, were similarly purified [9]. In view of these successes it seemed obvious that the same thing should be attempted with animal viruses. Beard, working with the very stable Shope papillomas, supplied material from which I obtained the virus in pure form [10]. Next I was able to concentrate the far less stable Western encephalomyelitis virus [11] which Ten Broek was growing in chicken embryonic tissue. Beard and I utilized this observation to prepare a highly effective formalinized vaccine against the disease [12].

Simon Flexner had retired as Director of the Rockefeller Institute at the time my ultracentrifugal experiments were starting. The full support he had always given was not continued by the new director and I was compelled to leave the Institute and take employment at the Lederle Laboratories. A serious epidemic of encephalomyelitis among horses in the United States was arrested by the several million doses of our vaccine [13] manufactured in my new laboratory. While there I had the opportunity to encourage the sale to American Cyanamid, owner of the Lederle Laboratories, of the first electron microscope manufactured by RCA. Then during a short stay in Ann Arbor in the midst of the Second World War, I found unused the #2 microscope; with it and a sister instrument Robley Williams and I developed metal evaporation [14] as a way to measure the heights of particles. The three-dimensional effects thus obtained led to preliminary shadowed micrographs of the tobacco mosaic virus.

At the National Institutes of Health in Bethesda when the war was over I was able to resume, with the electron microscope, the study of purified viruses that had been arrested a decade earlier. While examining preparations of tobacco mosaic virus [15] concentrated by ultracentrifugation, it was noted that their macromolecular rod-like particles were in strikingly regular quasi-crystalline arrangements. It happened that Bernal visited my laboratory as I was making the first of such electron micrographs. At his urging I took them to a meeting being organized to bring together in London electron microscopists from recently liberated Western Europe. At the time I made contact with Kenneth Smith, then Director of the Molteno Institute in Cambridge, who together with Roy Markham had been preparing single crystals of the tobacco necrosis virus protein. Replicas of these crystals showed for the first time the ordered arrangement of their particles on various faces of a single crystal [16]. Collaboration with Kenneth Smith continued for many years and extended to the insect viruses that were for him a major interest.

During the years that followed improved techniques of specimen preparation were used to visualize the particle arrangement on the faces of many other macromolecular crystals [17]. Crystalline arrays are now being seen in all sorts of biological material but at the outset it was very exciting, and I should add esthetically most satisfying, to see and not merely to deduce the way in which the elementary particles of a substance arrange themselves when crystallizing.

It is instructive to note that all the more interesting results of this work (the ultracentrifugal purification of viruses, the three-dimensional consequences of metal shadowing, the direct visualization of the molecular arrangement in crystals) were unforeseen products of the research rather than the fulfillment of its original intent.

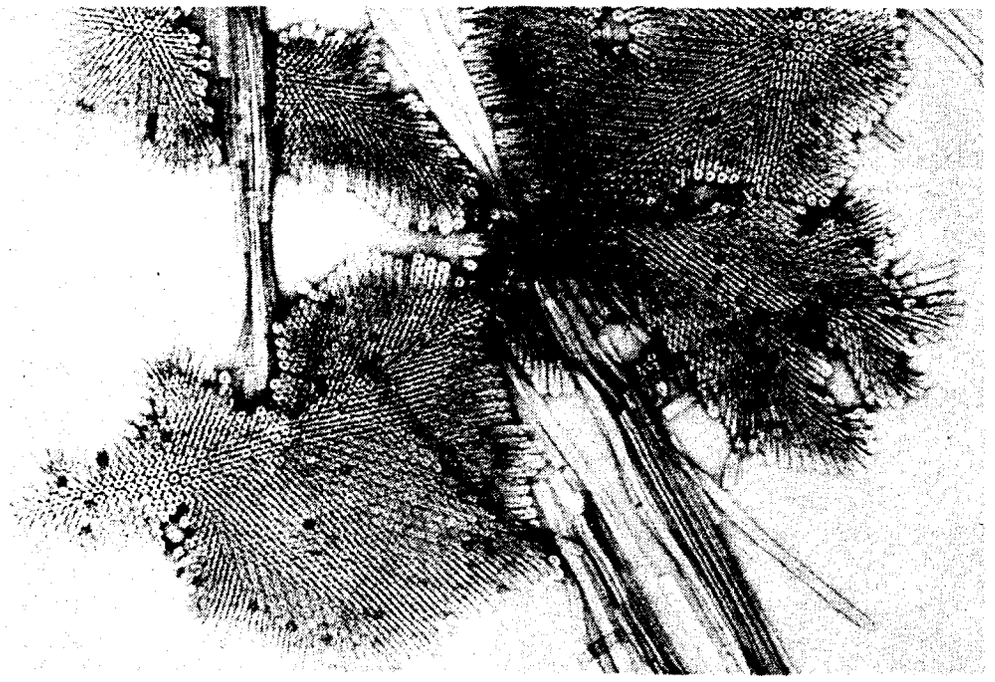

Fig. 2. A recent electron micrograph of tobacco mosaic virus rods. The rods are arranged in para-crystalline arrays with the 300 nM long axis of the virus particles vertical to the specimen plane. The central axial hole can be seen to advantage in particles photographed as end-on arrays. Several TMV rods can be seen in horizontal positions between the crystalline forms (× 110000). (Electromicrograph supplied by R. W. Horne, J. W. Harnden and R. Markham, John Innes Institute, Norwich, U.K.)

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Now in his 81st year Wyckoff was a pioneer in the use of X-rays to determine atomic positions in crystals, the development of the ultracentrifuge and the use of the electron microscope to study and visualise viruses and large molecules. He has been Professor of Physics at the University of Arizona since 1959, spending from 1959–1962 as Directeur de Recherche, at the Centre National de la Recherche Scientifique in France. From 1952–1954 he was Science Attache at the US Embassy in London and this was the first Embassy to be fitted with an electron microscope.

He is also the author of several books on crystallography, the electron microscope and most recently on the biochemistry of animal fossils.

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